HHS Public Access

Author manuscript

Haemophilia. Author manuscript; available in PMC 2015 December 09.

Published in final edited form as:

Haemophilia. 2014 May ; 20(0 4): 71–75. doi:10.1111/hae.12402.

RARE BLEEDING DISORDERS

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Abstract

Rare bleeding disorders (RBDs) are inherited deficiencies of coagulation factors as fibrinogen, Factor (F) FII, FV, FVII, combined FV/FVIII, FX, FXI, and FXIII. These disorders have usually a low prevalence in the general population and constitute approximately 3 to 5% of all coagulation disorders. However, in some countries they could have the same prevalence of hemophilia B due to the practice of consanguineous marriage. The clinical picture of RBDs are highly variable and could markedly vary from mild to severe, making either diagnosis and optimal treatment quiet challenging. This review focuses on 1) efforts to establish a bleeding assessment tool adequate to RBDs, 2) the optimal management of patient affected with FXI deficiency and 3) the correlation between clinical severity and laboratory diagnosis for determining the minimum coagulant activity required to prevent bleeding in each RBD.

Introduction

Rare bleeding Disorders (RBDs), representing 3–5% of all inherited coagulation factor deficiencies, include the inherited deficiencies of fibrinogen, FII, FV, FV+FVIII, FVII, FX, FXI and FXIII, generally transmitted in both sexes in autosomal recessive manner [1]. The prevalence of homozygous or double homozygous forms in general population vary from 1:500.000 for FVII deficiency to 1 in 2.000.000 for prothrombin and FXIII deficiency [1]. RBDs are characterized by a wide variety of symptoms from mild to severe which can vary significantly from one disorder to another, and from one patient to another even when suffering from the same type of disorder.

The clinical heterogeneity of RBDs associated with their rarity is a significant barrier to enhancing their deeper knowledge. Diagnosis, classification and adequate treatment of these disorders has been hampered by the variable clinical presentation and difficulty in recognizing affected patients, difficulty in collecting longitudinal clinical data and limits of laboratory assays.

Therefore a tool that could help us to diagnose and to predict the clinical severity pattern for each patient would be important. In the first part of this article, Dr. P. James from Queen's University, Kingston, Canada, will discuss the application of different bleeding assessment tools in RBDs.

In the second part, Prof. O. Salomon, from the University of Tel Aviv, Israel, will focus on treatment of patients affected with FXI deficiency, that unlike other coagulation factor deficiencies rarely presents spontaneous bleedings which on the contrary usually occur following surgery or trauma. This feature, together with the lack of correlation between clinical severity and plasmatic FXI coagulant levels, and the risk of thrombosis associated to replacement therapy, makes difficult the management of the patients.

Finally Dr. D. Mikovic, from the Blood Transfusion Institute of Serbia, will argue on the importance of finding a correlation between coagulant activity and clinical severity in RBDs to determine the hemostatic level of each single factor for preventing hemorrhage. A special mention will be paid to the importance of standardization of available coagulant assays.

Bleeding Assessment Tools – Rare Bleeding Disorders

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The accurate assessment of hemorrhagic symptoms is a key component in the diagnosis of bleeding disorders, including RBDs. However, the evaluation of bleeding symptoms is a well-recognized challenge for both patients and physicians, because the reporting and interpretation of bleeding symptoms is subjective. Significant symptoms may be overlooked because they are considered normal and minimal or trivial symptoms may be given undue consideration. The risk of this second issue is highlighted by the high frequency of bleeding symptoms reported by the general population [2,3]. In response to these challenges, a number of attempts have been made to standardize bleeding histories.

Over the years, multiple investigators have made attempts to standardize bleeding histories by identifying questions that best distinguish between affected and unaffected individuals. In 1990, Higham and colleagues published the PBAC (Pictorial Bleeding Assessment Chart) which allows women with heavy menstrual bleeding to track the number of pads or tampons used for a menstrual period as well as the degree of soiling [4]. Based on that information, a score is generated and PBAC scores ≥100 correlate with menorrhagia as defined as ≥80 mls of menstrual blood loss. In 1995, Sramek and colleagues published their experience with a bleeding questionnaire that was administered to patients known to have a bleeding disorder and a group of normal controls [5]. The most informative questions in terms of discrimination were about bleeding following traumatic events such as tonsillectomy or dental extraction (but not childbirth) and the presence of a bleeding disorder in a family member. In 2005, the International Society on Thrombosis and Haemostasis (ISTH) Scientific and Standardization Committee (SSC) on Von Willebrand factor (VWF) established a set of provisional criteria for the diagnosis of VWD type 1 including the threshold that must be met for mucocutaneous bleeding symptoms to be considered significant [6]. Over time, the field has increasingly focused on quantitative assessments of bleeding, and on the need for standardization.

Building on the ISTH provisional criteria, Rodeghiero et al developed and validated a BAT for the diagnosis of Type 1 VWD in a primarily adult population [7]. This bleeding questionnaire subsequently underwent a series of modifications, including one by Bowman et al specifically aimed at decreasing administration time [8] and culminating with the publication of the ISTH-BAT in 2010 [9]. Studies focused on evaluating the utility of these and other BATs for use in patients with RBD have begun. As a first step, a classification system for RBDs based on the association between coagulant factor activity and clinical bleeding severity was published by Peyvandi et al in 2012 [10].

By far the largest study to date was published by the European Network of Rare Bleeding Disorders (EN-RBD) Group [11]. The objective of this study was to explore the relationship between coagulation factor levels and bleeding severity in patients with RBDs using data on 489 patients registered with EN-RBD. Clinical bleeding episodes were classified into four categories of severity following consensus. Strong correlations were identified for deficiencies of fibrinogen, FX, FXIII and FV + VIII. Weaker correlations were identified for deficiencies of FV and FVII and no correlation for a deficiency of FXI.

Two papers specifically focused on issues facing women with bleeding disorders included patients with RBDs. The first, published by Kulkarni in 2006 included 14 women with FVII deficiency and 23 controls [12]. Women with FVII deficiency were more likely to have PBAC scores > 100 as well as anemia and had lower quality of life scores when compared with the controls. In the second paper, Siboni et al included a total of 228 subjects; 114 with bleeding disorders and 114 controls; 35 of the affected women had RBDs [13]. Their clinical assessment included administration of the PBAC as well as the Sramek bleeding score. Their analysis showed that affected women had a higher prevalence of excessive bleeding at menarche as well as menorrhagia and general bleeding symptoms. Additionally, in affected women, the bleeding score increased according to the severity of the coagulation factor defect although these results are very likely affected by the inclusion of women with VWD and carriers of hemophilia.

As mentioned, studies on patients with RBDs have been performed using the Condensed MCMDM1-VWD Bleeding Questionnaire and the ISTH-BAT. Tosetto and colleagues published a paper in 2011 evaluating the diagnostic utility of the Condensed MCMDM-1VWD Bleeding Questionnaire in 215 subjects referred for a possible bleeding disorder [14]. The performance of the BAT varied widely depending on the specific reason for referral (bleeding symptoms, family history or abnormal clotting test results).

One year later, Azzam and colleagues published a paper describing the diagnostic utility of the Condensed MCMDM-1VWD Bleeding Questionnaire to predict the presence of a bleeding disorder in 30 women with unexplained menorrhagia between the ages of 11 and 31 years [15]. Overall, a high proportion of women enrolled (20/30 or 66.6%) had an underlying bleeding disorder reflecting the fact that they recruited from a referral population. Although they reported a sensitivity of 85%, specificity of 90%, positive predictive value = 0.89 and negative predictive value of 0.86 only three patients in the study had RBDs (one each with deficiency of fibrinogen, FV and FV + VIII) making it impossible to generalize the results to all RBDs.

Shapiro et al published a description of the clinical and laboratory features of 35 patients with hereditary dysfibrinogenemia; bleeding symptoms were evaluated using the ISTH-BAT [16]. Of the 35, 22 (63%) had at least one bleeding symptom identified. Three (9%) had thrombosis and overall the bleeding scores did not differ from matched healthy controls.

In total, this review includes discussion of the use of BATs in 594 patients with RBDs, a reasonable start given the overall disease prevalence of 0.5 – 2 per million. Additional study is warranted however, in order to address the critical question of the ideal BAT for RBDs. Many of the tools discussed in this review were originally designed for more common bleeding disorders, such as VWD and it is currently not known if they provide the optimal assessment of these patients. International collaborative efforts are required to design and carry out the necessary studies to answer this question. As an initial step, the WFH is presently carrying out a project to review and catalogue the existing bleeding questionnaires including plans to highlight the strengths and weaknesses of the existing tools.

New concepts in therapy of congenital factor XI deficient patients

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FXI deficiency is a rare bleeding disorder which is distinct from other coagulation factor deficiencies since bleeding usually occurs following surgery or trauma, and sometimes only after scar tissues are detached. Severity of bleeding does not correlate with FXI levels, and replacement therapy may be associated with risk of thrombosis [17].

The main concern when treating patients with severe FXI deficiency is excessive prophylactic treatment administered to non-bleeding patients, and perhaps inadequate therapy with respect to the bleeding patients. Unfortunately, to date, there is no bleeding score available for FXI deficient patients to estimate quantity, extent of bleeding and its correlation with bleeding risk in upcoming procedures; furthermore, the current approach is not applicable to patients with no prior surgeries. The role of thrombophilia, levels of VWF, FVIII, fibrinogen, thrombomodulin, as well as platelet counts as “bleeding modifiers” in the context of FXI deficiency is still unknown. However, gene-environmental interaction is a phenotype modifier of FXI deficiency under conditions such as pregnancy [18,19], sepsis. Yet thrombin generation test (TGT), which is one of the global assays used to assess overall haemostasis in a given patient, seems to distinguish between bleeders and non-bleeders through peak height [20,21]; however this data needs further standardisation. Furthermore, it was recently shown that patients with history of bleeding exhibited reduced fibrin network density in comparison to non-bleeders when assessed by laser scanning confocal microscopy [22]. In the meantime, in the absence of sensitive and standardized laboratory method for assessment of bleeding risk in severe FXI deficient patients, prophylactic treatment for invasive procedures is required prior to surgery regardless of the history of bleeding.

Nowadays the commonly treatment offered to patients with severe FXI deficiency is fresh frozen plasma (FFP) at the dose of 15 mL/kg targeting FXI activity of 40% for approximately a week [17]. The treatment is associated with potential complications such as

volume overload in patients with congestive heart failure and renal failure. In addition, FFP cannot be used in patients with inhibitors to FXI, and may enhance the risk of inhibitor development following exposure in those with undetectable levels of FXI [23]. The use of FFP can be complicated by increasing the risk of transfusion-transmitted diseases, allergic reactions and even anaphylactic shock, especially in those with immunoglobulian A (IgA) deficiency when IgA-depleted FFP is not applied.

FXI concentrates (plasma derived heat-treated) in the absence of recombinant FXI is another option offered in some countries. It is efficient in predicting increment of FXI levels, and since it has a long half-life, this treatment can be given on alternate days. The target level should be 30–40 IU/dL. But the caveat with their use is that both currently available products (Bio Products laboratory – United Kingdom and LFB Biomedicaments – France) have been associated with thrombosis even after adding heparin to antithrombin in the BPL product and antithrombin and heparin to C1 esterase in the LFB product [17,24,25]. Furthermore, patients with undetectable levels of FXI in plasma are at risk of developing inhibitors following exposure to the concentrates [26], and they cannot be used in IgA deficient patients. Thus before it is prescribed for use, screening for antibodies is mandatory in patients with undetectable FXI levels who were previously exposed to FFP, FXI concentrates or immunoglobulin.

Low-dose (15–30 μg/kg) recombinant factor VIIa (rFVIIa), a bypassing agent, has been successfully used in patients with severe FXI deficiency both with and without inhibitors [27,28]. Caution is required when used at higher doses, like those regularly used to treat hemophilia A and B, because of the increased risk of thrombosis [29,30]. It is the only treatment available for patients with inhibitors, and has been recently suggested to be used as primary treatment to avoid exposure to blood product.

Antifibrinolytic agents e.g. tranexamic acid, 6-aminocaproic acid are currently used for minor procedures as monotherapy, or in combination with low-dose rFVIIA or FFP in major procedures.

Altogether, before planning prophylactic treatment for patients with severe FXI deficiency, the following issues must be addressed:

1. Site and type of surgery [31]
2. Presence of an inhibitor
3. Combined hemostatic defects
4. Thrombotic risk
5. Volume overload
6. Presence of IgA deficiency
7. Previous exposure/lack of exposure to blood products
8. Environmental interactions

Taken together, tailored therapy according to the individual risk and type of procedure is the treatment to aspire for when managing FXI deficient patients. It remains to be established whether one of the global coagulation tests, including assays of fibrinolysis and/or clot structure, will eventually efficiently predict the bleeding risk of a given individual before innovative prophylactic treatment can be recommended.

Laboratory *versus* phenotypic association in Rare Bleeding Disorders

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The natural history of RBDs is characterized by lifelong bleeding tendency. Clinical presentation is highly variable ranging from a mild or moderate to severe form with serious or life-threatening bleeding episodes and therefore the bleeding risks in affected individuals may be difficult to assess [1,32].

It contrast to haemophilia, where FVIII or FIX level <1% is usually associated with spontaneous and frequent joint bleeding episodes while patients with >5% remain largely asymptomatic; there is a heterogeneous association between residual plasma coagulant factor activity and clinical bleeding severity in different RBDs. The assays and regents used to measure coagulation factor level should to be considered, because there are usually significant inter-laboratory differences in the results of factor assays [10,32].

Despite efforts in research into RBDs, knowledge gaps still remain, and randomized controlled studies may be difficult due to sample size and length of follow-up limitations. These limitations underline the need to develop an accurate data collection tool, available to the centers around the world that would enable longitudinal and follow up data collection. Patient registries, national and international, are powerful tools with considerable potential for rare disease research [33].

The European Network of Rare Bleeding Disorders (EN-RBD) was established to bridge the gap between knowledge and practice in the care of patients with RBDs [11]. The EN-RBD project, coordinated by the University of Milan, has involved 13 European treatment centers from 11 countries. Results of data analyses on 489 patients registered in the EN-RBD were reported [11]. Abnormal bleeding episodes from mucous membranes (oral cavity bleeding, epistaxis, menorrhagia) are the most frequent bleeding manifestations in RBDs [10]. Abnormal bleeding from the skin and prolonged bleeding after trauma, invasive procedure and surgery are frequent symptoms [10]. The most severe bleeding symptoms are found in patients with afibrinogenemia, FX deficiency, and FXIII deficiency, with a relatively high frequency of spontaneous major bleeding in joints and muscles. Gastro intestinal tract bleeding and central nervous system bleeding are relatively rare for all defects, except for FX deficiency [9]. Umbilical cord bleeding, typical of afibrinogenemia and FXIII deficiency, are relatively frequent also in prothrombin, FV and FX deficiency [10].

According to the results it is evident that it is not appropriate to use a single criterion of classification for all types of RBDs [10]. There was a strong association between coagulation factor activity level and clinical bleeding severity for fibrinogen, FX and FXIII [10,11]. Thus, patients with low coagulant activity levels of corresponding factor had high

occurrence of spontaneous major bleeding, while patients with sufficient activity remained asymptomatic. A weaker association was present for FV and FVII deficiencies [10,11]. There was no association between coagulation factor activity level and clinical bleeding severity for FXI i.e. FXI coagulation factor activity do not predict clinical bleeding severity [10,11]. For FII deficiency sample was too small to draw any correlation [11]. The lack of association between coagulation factor activity level and bleeding severity in patients with RBDs may be attributed to the potential role of other factors in determining bleeding severity, like platelets and fibrinolytic potential. The observed coagulation factor activity levels necessary to ensure complete absence of bleeding episodes and coagulation factor activity levels that correspond with probability of major spontaneous bleeding show high diversity in the different rare coagulation deficiencies.

The template of the EN-RBD database has proven to be a valuable tool for the extrapolation of information relevant to clinical practice and further validation of a bleeding risk-assessment. A more detailed evaluation on each single factor deficiency is necessary. Prospective data collection on patients with RBDs (PRO-RBDD) project has been established with the aim to increase the knowledge on clinical and therapeutic aspects of these disorders. Some consensus on factor assay methodology is important so that values from different laboratories/centers can be compared as well as further research in potential role of global coagulation assays in accurate prediction of the hemorrhagic risk.

References

1. Peyvandi F, Palla R, Menegatti M, Mannucci PM. Rare Bleeding Disorders: General Aspects of Clinical Features, Diagnosis, and Management. Semin Thromb Hemost. 2009; 35:349–355. [PubMed: 19598063]
2. Sadler JE. Von Willebrand disease type 1: a diagnosis in search of a disease. Blood. 2003; 101:2089–93. [PubMed: 12411289]
3. Mauer AC, Khazanov NA, Levenkova N, et al. Impact of sex, age, race, ethnicity and aspirin use on bleeding symptoms in healthy adults. J Thromb Haemost. 2011; 9:100–8. [PubMed: 20942847]
4. Higham JM, O'Brien PM, Shaw RW. Assessment of menstrual blood loss using a pictorial chart. Br J Obstet Gynaec. 1990; 97:734–9.
5. Srámek A, Eikenboom JC, Briët E, et al. Usefulness of patient interview in bleeding disorders. Arch Inter Med. 1995; 155:1409–15.
6. Sadler JE, Rodeghiero F. Provisional criteria for the diagnosis of VWD type 1. J Thromb Haemost. 2005; 3:775–7. [PubMed: 15842361]
7. Rodeghiero F, Castaman G, Tosetto a, et al. The discriminant power of bleeding history for the diagnosis of type 1 von Willebrand disease: an international, multicenter study. J Thromb Haemost. 2005; 3:2619–26. [PubMed: 16359502]
8. Bowman M, Mundell G, Grabell J, et al. Generation and validation of the Condensed MCMDM-1VWD Bleeding Questionnaire for von Willebrand disease. J Thromb Haemost. 2008; 6:2062–6. [PubMed: 18983516]
9. Rodeghiero F, Tosetto a, Abshire T, et al. ISTH/SSC bleeding assessment tool: a standardized questionnaire and a proposal for a new bleeding score for inherited bleeding disorders. J Thromb Haemost. 2010; 8:2063–5. [PubMed: 20626619]
10. Peyvandi F, Di Michele D, Bolton-Maggs PHB, et al. Classification of rare bleeding disorders (RBDs) based on the association between coagulant factor activity and clinical bleeding severity. J Thromb Haemost. 2012; 10:1938–43. [PubMed: 22943259]

11. Peyvandi F, Palla R, Menegatti M, et al. Coagulation factor activity and clinical bleeding severity in rare bleeding disorders: results from the European Network of Rare Bleeding Disorders. J Thromb Haemost. 2012; 10:615–21. [PubMed: 22321862]
12. Kulkarni A, Lee CA, Griffeon A, et al. Disorders of menstruation and their effect on the quality of life in women with congenital factor VII deficiency. Haemophilia. 2006; 12:248–52. [PubMed: 16643209]
13. Siboni SM, Spreafico M, Calò L, et al. Gynaecological and obstetrical problems in women with different bleeding disorders. Haemophilia. 2009; 15:1291–9. [PubMed: 19664014]
14. Tosetto, a; Castaman, G.; Plug, I., et al. Prospective evaluation of the clinical utility of quantitative bleeding severity assessment in patients referred for hemostatic evaluation. J Thromb Haemost. 2011; 9:1143–8. [PubMed: 21435168]
15. Azzam HAG, Goneim HR, El-Saddik AM, et al. The condensed MCMDM-1 VWD bleeding questionnaire as a predictor of bleeding disorders in women with unexplained menorrhagia. Blood Coag Fibrinolysis. 2012; 23:311–5.
16. Shapiro SE, Phillips E, Manning RA, Morse CV, Murden SL, Laffan MA, Mumford AD. Clinical phenotype, laboratory features and genotype of 35 patients with heritable dysfibrinogenaemia. Br J Haematol. 2013; 160:220–7. [PubMed: 23061815]
17. Duga S, Salomon O. Congenital factor XI deficiency: an update. Semin Thromb Hemost. 2013; 39:621–31. [PubMed: 23929304]
18. Salomon O, Steinberg DM, Tamarin I, et al. Plasma replacement therapy during labor is not mandatory for women with severe factor XI deficiency. Blood Coagul Fibrinolysis. 2005; 16:37–41. [PubMed: 15650544]
19. Myers B, Pavord S, Kean L, et al. Pregnancy outcome in Factor XI deficiency: incidence of miscarriage, antenatal and postnatal haemorrhage in 33 women with Factor XI deficiency. BJOG. 2007; 114:643–6. [PubMed: 17439571]
20. Rugeri L, Quélin F, Chatard B, et al. Thrombin generation in patients with factor XI deficiency and clinical bleeding risk. Haemophilia. 2010; 16:771–777. [PubMed: 20398070]
21. Livnat T, Martinowita U, Mansharov U, Salomon O. Thrombin generation test can predict bleeding tendency in patients with severe factor XI deficiency. ASH –abstract. 2013
22. Zucker M, Salomon O, Seligsohn U, Wolberg AS. Abnormal plasma clot structure and stability distinguish bleeding risk in patients with severe factor XI deficiency. ISTH. 2013 abstract No. 1227.
23. Salomon O, Zivelin A, Livnat T, et al. Prevalence, causes, and characterization of factor XI inhibitors in patients with inherited factor XI deficiency. Blood. 2003; 101:4783–4788. [PubMed: 12586617]
24. Mannucci PM, Bauer KA, Santagostino E, et al. Activation of the coagulation cascade after infusion of a factor XI concentrate in congenitally deficient patients. Blood. 1994; 15:1314–1319. [PubMed: 8049446]
25. Boehlen F, Casini A, Pugin F, de Moerloose P. Pulmonary embolism and fatal stroke in a patient with severe factor XI deficiency after bariatic surgery. Blood Coagul Fibrinolysis. 2013; 24:347–50. [PubMed: 23187784]
26. Quélin F, Trossaërt M, Sigaud M, Mazancourt PD, Fressinaud E. Molecular basis of severe factor XI deficiency in seven families from the west of France. Seven novel mutations, including an ancient Q88X mutation. J Thromb Haemost. 2004; 2:71–6. [PubMed: 14717969]
27. Livnat T, Tamarin I, Mor Y, et al. Recombinant activated factor VII and tranexamic acid are haemostatically effective during major surgery in factor XI-deficient patients with inhibitor antibodies. Thromb Haemost. 2009; 102:487–492. [PubMed: 19718468]
28. Riddell A, Abdul-Kadir R, Pollard D, et al. Monitoring low dose recombinant factor FVIIa therapy in patients with severe factor XI deficiency undergoing surgery. Thromb Haemost. 2011; 106:521–527. [PubMed: 21833443]
29. Schulman S, Nemeth G. An illustrative case and a review on the dosing of recombinant factor VIIa in congenital factor XI deficiency. Hemophilia. 2006; 12:223–227.
30. O'Connell NM, Riddell AF, Pascoe G, et al. Recombinant factor VIIa to prevent surgical bleeding in factor XI deficiency. Haemophilia. 2008; 14:775–781. [PubMed: 18384352]

31. Salomon O, Steinberg DM, Seligshon U. Variable bleeding manifestations characterize different types of surgery in patients with severe factor XI deficiency enabling parsimonious use of replacement therapy. Haemophilia. 2006; 12:490–493. [PubMed: 16919078]

32. Bolton Maggs PHB, Perry DJ, Chalmers EA. The rare coagulation disorders – review with guidelines for management from the United Kingdom Haemophilia Centre Doctors' Organisation. Haemophilia. 2004; 10:593–628. [PubMed: 15357789]

33. Peyvandi F, Spreafico M. National and International registries of rare bleeding disorders. Blood Transfusion. 2008; 6(Suppl2):s45–s48. [PubMed: 19115503]